September 25, 1949.

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Dear Lu-

I am returning Davis' mss, with my own reactions. I would appreciate having the opportunity to study them more closely again later.

It really has to be left to you as the editor to decide what should be kept in, and I have no objections to the retention of any or all of it. My MS was written as concisely as possible, and as an outline of methods, rather than of theoretical substance. I also tried to emphasize the aspects that might be useful in genetic investigations, but I did not get the impression that this was the place for a review of bacterial genetics or biochemistry.

The points that Bernie raised that I would be particularly anxious to see brought in are; p. 6)use of thiosulfate (and thioglycollate too!) for sulfide mutants. p. 10) the purity of agar Tables) corrected concentration ranges for vitamins, when these are known. Also, Davis' point concerning a mention ofmutants with partial requirements is something that I am sorry to have overlooked. However, such mutants are especially treacherous for genetic work. The mention of them should be in the text, but as Bernie pointed it out, perhaps he should have the opportunity of bringing it up as comment. I also think that his insertion on his improvements of the penicillin method should be included if it can be abbreviated to about a page. He has a fair amount of interesting but unessential comment.

Bernie also included a fair amount of nutritional information which is of great interest. My aim in writing this section was to give enough detail about methods, and what might be expected to be found to carry a new investigator into the field, but not to provide him with all the biochemical information needed to lead him out again. I think that a symposium on microbial nutrition, incorporating some of the viewpoints that Davis and Snell have provided, is badly needed, but I hope that a place can be found for such a symposium where there would be space for a complete discussion.

Now for a point-by-point discussion.

p. 1) "Auxotrophs" The term has been used by Lwoff in this sense, but at CSH 1946 (see Symp. V. 11) "heterotroph" was adopted. These terms still describe only the nutrition; one could say heterotrophic mutant synonymously with nutritional mutant. The issue might be worth three words in a parenthesis. I don't think that a methods book is the place for semantic innovations.

p, 4) "marginal"- I don't care. "Limiting" rather than "limited" enrichment might be better, and this is what I wanted to bring out with "marginal". No further comment

p. 6) N. F. C.

p. 9) L-valine. Mentioned only because it is important when the commercially available product is used.

Steroids and carotenoids are certainly found in microbial protoplasm and might be expected to be found eventually as growth factors. Sterols are required by some flagellates (Lwoff?) and Ottke (Yale) once thought to have had a sterol-dependent Neurospora. Probably more such mutants will be found if the "complete" media used are properly supplemented.

p. 10) OK. Concur in fact and emphasis

p. 11) Published examples were cited.

p. 13) Syntrophism describes the ability of a mixed culture to grow where its components will not. As such it is a useful quasi-genetic test. I neither endorse nor object to including further details of biochemical interest

p. 14) I don't know the details of the EMB test, but the dark reaction is certainly not due to killing. However, the green sheen which is inappropriately used to distinguish E. coli from Aerobacter might conceivably depend on killing, although I still don't think so. The reaction probably depends upon the precipitation of a methylene blue eosinate at the effective isoelectric point of the complex.

p. 16) May belong in the nutrition symposium??

Tables: water of cryst.: Yes, but it doesn't matter.

Vitamin levels: rather arbitrary, and improve them if you can. But E. coli is not the only bacterium. The detailed listing of mutants does not, I think really belong here,

Leaky mutant(s): an excellent suggestion, as mentioned.

I don't know whether it would be appropriate to bring in the "reversion test" for double mutants in this place. Like many other points that were raised, this is well covered in his Experientia paper, to which reference should be made.

Precisely because hydroxyproline requiring mutants have never been found, this compound should be tested routinely on amino acid mutants. I agree about norleucine and norvaline, but it wouldn't hurt to try them for protein hydrolysate mutants which do not respond to recognized amino acids. But a parenthesis (probably not naturally occurring amino acids) should be added.

I don't envy you your editorial responsibilities at this point. Davis has raised a good many interesting and pertinent points, but do they belong in this publication? If they do, I think he would have done better to write ~~the section.~~ another section on the application of genetics to biochemistry.

Sincerely,